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N-Nitroso Compounds in the Gastric Juice of Normal Controls, Patients With Partial Gastrectomies, and Gastric Cancer Patients

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Background: It has been suggested that the variation of biochemical and microbiological parameters in the gastric juice may play a role in the development of gastric cancer. In the present study we concurrently assessed the presence of N-Nitroso compounds (NOC) and their precursors, bacteria and carcinoembryonic antigen (CEA) in the gastric juice of normal controls, patients with gastric resection, and advanced gastric cancer.
Methods: Detailed analyses of biochemical and microbiological parameters such as pH, nitrite (NO_2) concentration, N-nitroso compounds (NOC) concentration, carcino-embryonic antigen (CEA) level, total viable counts (TVC), nitrate-reductase positive bacterial counts (NRPBC), and identification of micro-organisms were carried out.
Results: Significantly higher mean pH values, NO_2, NOC and CEA concentrations, TVC, and NRPBC were found in partial gastrectomies compared with normal controls, and all these intragastric parameters were significantly higher in patients with gastric cancer than in those with partial gastrectomies. As far as surgical methods are concerned, higher mean pH values, NO_2 and NOC concentrations, TVC, NRPBC, and anaerobic bacterial counts were observed in the juice of patients with Billroth II compared with Billroth I gastrectomies. Apart from the type of surgical reconstruction, higher mean NOC levels were recorded in patients with more severe histological changes and *H. pylori* infection.
Conclusions: All these data suggest that the presence of high levels of NOC in the gastric juice of gastroresected patients can be considered a risk factor of gastric stump cancer.

KEY WORDS: **gastric cancer, partial gastrectomy, bacteria, N-nitroso compounds, carcinoembryonic antigen**

INTRODUCTION

Adenocarcinoma of the gastric stump (GSC) is a recognized late complication of partial gastrectomy performed for benign peptic ulcer. Several reports [1–3] have suggested that Billroth II gastrojejunostomy carries a higher cancer risk than Billroth I gastroduodenostomy, whereas others have found no significant differences between these two operations [4]. Only two studies showed a significantly higher incidence of gastric stump cancer after a Billroth II resection [5,6]. Several factors have been correlated with the development of GSC, including primary disease, patient's age at the time of surgery, type of reconstructive technique, length of the latent period, and preoperative gastric histology [7]. Caygill et al. [8] reported that Billroth II partial gastrectomy performed 15 years before or longer, was significantly correlated with

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TABLE I. Age (years) and Sex (M, F) Distribution of 79 Selected Subjects

	n	Total	Age (years) <35	36–45	46–55	56–65	>65	Mean age ± SD
Normal controls	20	9M, 11F			9M, 8F	3F		50.7 ± 4.0
Billroth I	23	14M, 9F		3M, 2F	6M, 2F	5M, 3F	2F	51.7 ± 8.2
Billroth II	22	12M, 10F	1F	4M, 2F	4M, 2F	2M, 4F	2M, 1F	52.0 ± 11.8
Gastric cancers	14	8M, 6F		1F	8M, 3F	2F		51.9 ± 5.6

TABLE II. Significant Correlations Among Continuous Variables in 79 Gastric Juice Samples

Dependent variable[a]	Independent variable[a]	β	Standard error of β	t	r	*P*
NO_2	pH	5.095	0.650	7.834	0.665	0.0001
NO_2	TVC	2.884	0.502	5.738	0.547	0.0001
NO_2	NRPBC	2.480	0.515	4.811	0.481	0.0001
TVC	pH	1.116	0.105	10.563	0.769	0.0001
NRPBC	pH	0.958	0.129	7.425	0.646	0.0001
NRPBC	TVC	0.954	0.041	22.996	0.934	0.0001
NOC	pH	0.021	0.009	2.360	0.259	0.0208
NOC	NO_2	0.005	0.001	4.962	0.492	0.0001
NOC	TVC	0.030	0.005	5.504	0.531	0.0001
NOC	NRPBC	0.028	0.005	5.243	0.512	0.0001
CEA	NOC	107.350	21.130	5.079	0.500	0.0001
CEA	pH	7.372	1.837	4.012	0.416	0.0001
CEA	NO_2	1.322	0.212	6.098	0.571	0.0001
CEA	TVC	4.671	1.254	3.724	0.454	0.0004

NO_2 = nitrite; TVC = total viable bacterial counts; NRPBC = nitrate-reductase positive bacterial counts; NOC = N-nitroso compounds; CEA = carcino-embryonic antigen.

an increased risk for developing GSC. This has been attributed to several pathogenetic factors but particularly to enterogastric and pancreaticobiliary reflux, hypochlorhydria, action of microflora, and formation of N-nitroso compounds [9].

The present study was prompted by the contradictory results reported in the literature. The presence of N-nitroso compounds (NOC), their precursors, bacteria, and carcino-embryonic antigen (CEA) in the gastric juice of patients with gastric resection, normal controls, and advanced gastric cancer (GC) patients has been investigated only by few researchers and never concurrently.

MATERIALS AND METHODS

Patients

Seventy-nine subjects (Table I) were selected for this study including 23 patients who have had a Billroth I gastrectomy with end-to-end anastomosis (BI) and 22 patients who have had a Billroth II resection by the Reichel-Polya technique (BII). All partial gastrectomies were performed for duodenal ulcer 8–20 years previously. There were 20 normal controls selected on the basis of histological normalcy from a group of 34 asymptomatic volunteers found to be free of gastric lesions at endoscopy. Fourteen patients had gastric cancer, 10 with nonintestinal, diffuse, histological type, and 4 with intestinal type adenocarcinoma according to Lauren's classification [10].

Histological, Microbiological, and Biochemical Analyses

During the week preceeding the examination, the subjects were given no drugs. After an overnight fast for at least 12 hours, endoscopy was performed, during which multiple gastric biopsies and a gastric juice sample were obtained. The endoscope and its channel were disinfected with activated glutaraldehyde, and the reagent was removed using nitrite-free sterile water. The gastric juice was drawn through a sterilized polyethylene catheter passed through the biopsy channel of the instrument in order to avoid contamination with saliva and bile. The biopsy specimens were taken from the gastric stump around the anastomosis to evaluate the histology and the *Helicobacter pylori* status. These specimens were routinely processed, embedded in paraffin wax, and stained by haematoxilin and eosin, and the Gimenez method for *H. pylori* status. The histological changes were evaluated according to Watt et al. [11] and divided into two groups. Chronic superficial gastritis, cystic dilatation of glands, focal chronic atrophic gastritis, and complete intestinal metaplasia were classified as the lower degree lesion group. Multifocal atrophic gastritis, uncompleted intestinal metaplasia, and dysplasia were classified as the higher grade lesion group.

pH, NO_2, NOC, and CEA concentrations were evalu-

TABLE III. Significant Correlations Among Continuous Variables in 79 Gastric Juices Subdivided Into Groups*

BI and BII (n = 45)						
Dependent variable	Independent variable	β	Standard error of β	t	r	*P*
NO_2	pH	7.566	1.766	4.285	0.547	0.0001
NOC	TVC	0.025	0.009	2.620	0.372	0.011
NOC	NRPBC	0.019	0.008	2.399	0.343	0.02
pH	Time since surgery	0.172	0.054	3.195	0.437	0.003
BI (n = 23)						
Dependent variable	Independent variable	β	Standard error of β	t	r	*P*
NO_2	pH	12.224	4.171	2.931	0.230	0.008
TVC	pH	1.655	0.558	2.968	0.544	0.007
NRPBC	TVC	0.993	0.112	8.566	0.882	0.0001
BII (n = 22)						
Dependent variable	Independent variable	β	Standard error of β	t	r	*P*
NOC	pH	−0.097	0.030	−3.199	−0.580	0.0045
NRPBC	TVC	1.124	0.171	6.572	0.827	0.0001
CEA	TVC	5.189	1.828	2.838	0.536	0.01
pH	Time since surgery	0.178	0.063	2.806	0.531	0.01
Gastric cancer (n = 14)						
Dependent variable	Independent variable	β	Standard error of β	t	r	*P*
NOC	NO_2	0.009	0.004	2.277	0.549	0.041
CEA	NO_2	2.549	0.794	3.212	0.680	0.008
NOC	TVC	0.086	0.030	2.880	0.639	0.013
NRPBC	TVC	1.200	0.170	7.172	0.900	0.0001

*NO_2 = nitrite; NOC = N-nitroso compounds; TVC = total viable bacterial counts; NRPBC = nitrate-reductase positive bacterial counts; CEA = carcino-embryonic antigen.

ated in the gastric juice and total viable bacterial counts (TVC), nitrate reductase-positive bacterial counts (NRPBC), and individual bacterial species were identified. pH was evaluated using a glass electrode, NO_2 and NOC were assayed by the Walters et al. method [12], the analyses being performed at the Leatherhead Food Research Laboratory (UK) within a month from the sampling procedure. CEA was assessed as described by Castelli et al. [13] and the microbiological analyses were performed by the method described by Carboni et al. [14].

Statistical Analysis

Correlation coefficients (Pearson's r or Spearman's r_s) and linear regression parameters were estimated for continuous variables. Differences among mean values of continuous variables in patient categories were analysed by Scheffe's procedure of the analysis of variance. To analyse the differences between the means of two groups of patients, the t test (applying the Cochran procedure when necessary), or the Wilcoxon test when necessary on account of group size, were employed. Contingency tables of categorical variables were evaluated by the Chi-square test or Fisher's exact test (for expected variables <5). All *P* values are two-sided and considered significant if <0.05. The SAS statistical package was used for all analytical procedures.

RESULTS

The mean age of the four groups of patients was not significantly different (Table I). The mean age (±SD years) since gastric resection of BI type (12.6 ± 2.1) was not significantly different ($t = 0.67$, NS) from the BII operations (13.2 ± 3.5).

The relationships and associations among continuous variables were analysed in all subjects (n = 79). Table II shows the significant correlations that emerged from this analysis. Significant correlations decreased when the relationships and the associations among the continuous variables were analysed in each group of subjects (Table III). This occurred particularly in normal controls, where no coefficient of correlation was significant. The study of resected patients as a whole revealed a significant correlation between an increase in time since the operation and an increase in pH of gastric juice. In BII resections there was a negative correlation between an increase in pH of the gastric juice and NOC levels.

Mean pH Values, NO_2, NOC and CEA concentrations, TVC, and NRPBC growth were found to be significantly

TABLE IV. Mean Values (± S.D.) for pH, Nitrite (NO_2), N-nitroso Compounds (NOC), Carcino-embryonic Antigen Level (CEA), Total Viable Bacterial Counts (TVC), and Nitrate-positive Bacterial Counts (NRPBC) in 79 Gastric Juice Samples†

	n	pH	NO_2 μmol/l	NOC μmol/l	CEA ng/ml	TVC $\log_{10}$ org./ml	NRPBC $\log_{10}$ org./ml
Normal controls	20	2.04 ± 0.78 *,**	1.67 ± 2.05 *,**	0.011 ± 0.02 *,**	3.16 ± 3.26 *,**	0.10 ± 0.45 *,**	0.0 ± 0.0 *,**
Partial gastrectomies	45	6.47 ± 1.12 *,***	20.27 ± 15.57 *,***	0.120 ± 0.16 *,***	27.67 ± 19.52 *,***	5.77 ± 2.33 *	4.95 ± 2.78 *
Gastric cancers	14	7.31 ± 0.74 **,***	33.93 ± 16.64 **,***	0.268 ± 0.28 **,***	86.12 ± 62.39 **,***	6.94 ± 2.10 **	6.37 ± 2.79 **

*Significant difference mean ($P < 0.05$) between normal controls and partial gastrectomies.
**Significant difference mean ($P < 0.05$) between normal controls and gastric cancers.
***Significant difference mean ($P < 0.05$) between partial gastrectomies and gastric cancers.
†NO_2 = nitrite; NOC = N-nitroso compounds; CEA = carcino-embryonic antigen; TVC = total viable bacterial counts; NRPBC = nitrate-reductase positive bacterial counts.

TABLE V. Significant Differences Among Mean Values of Parameters in the Four Groups of Gastric Juices (Scheffe's Procedure)

Parameters[a]	Comparison among groups[b] (mean ± SD)	Difference among means (95% confidence intervals)
pH	GC (7.31 ± 0.64) > B I (5.92 ± 0.75)	1.39 (0.53–2.24)
	GC (7.31 ± 0.64) > NC (2.04 ± 0.78)	5.27 (4.39–6.15)
	B II (7.05 ± 1.18) > B I (5.92 ± 0.75)	1.12 (0.37–1.88)
	B II (7.05 ± 1.18) > NC (2.04 ± 0.78)	5.01 (4.23–5.79)
	B I (5.92 ± 0.75) > NC (2.04 ± 0.78)	3.88 (1.11–4.66)
NO_2 (μmol)	GC (33.93 ± 16.64) > B I (14.36 ± 17.11)	19.55 (6.95–32.19)
	GC (33.93 ± 16.64) > NC (1.67 ± 2.05)	32.26 (19.29–45.24)
	B II (26.45 ± 11.1) > B I (14.36 ± 17.11)	12.1 (0.99–23.2)
	B II (26.45 ± 11.1) > NC (1.67 ± 2.05)	24.8 (13.28–36.25)
	B I (14.36 ± 17.11) > NC (1.67 ± 2.05)	12.7 (1.31–24.08)
NOC (μmol/l)	GC (0.268 ± 0.28) > B I (0.062 ± 0.08)	0.20 (0.09–0.31)
	GC (0.268 ± 0.28) > NC (0.011 ± 0.02)	0.25 (0.14–0.37)
	B II (0.176 ± 0.19) > B I (0.062 ± 0.08)	0.11 (0.01–0.21)
	B II (0.176 ± 0.19) > NC (0.011 ± 0.02)	0.16 (0.06–0.26)
CEA (ng/ml)	GC (86.12 ± 62.39) > B I (29.67 ± 20.21)	56.45 (27.39–85.65)
	GC (86.12 ± 62.39) > B II (25.59 ± 19.04)	60.53 (31.22–89.85)
	GC (86.12 ± 62.39) > NC (3.16 ± 3.26)	82.96 (53.09–112.84)
TVC ($\log_{10}$ org./ml)	GC (6.94 ± 2.10) > B I (4.81 ± 2.30)	2.12 (0.33–0.92)
	GC (6.94 ± 2.10) > NC (0.10 ± 0.45)	6.84 (4.99–8.69)
	B II (6.78 ± 1.96) > B I (4.81 ± 2.30)	1.97 (0.39–3.55)
	B II (6.78 ± 1.96) > NC (0.10 ± 0.45)	6.68 (5.04–8.32)
	B I (4.81 ± 2.30) > NC (0.10 ± 0.45)	4.71 (3.09–6.33)
NRPBC ($\log_{10}$ org./ml)	GC (6.37 ± 2.79) > B I (3.99 ± 2.59)	2.37 (0.13–4.60)
	GC (6.37 ± 2.79) > NC (0 ± 0)	6.37 (4.07–8.66)
	B II (5.96 ± 2.67) > NC (0 ± 0)	5.96 (3.93–8.00)
	B I (3.99 ± 2.59) > NC (0 ± 0)	3.10 (1.98–6.01)
	B II (5.96 ± 2.67) > B I (3.99 ± 2.59)	1.97 (0.10–3.93)

[a]NO_2 = nitrite; NOC = N-nitroso compounds; CEA = carcino-embryonic antigen; TVC = total viable bacterial counts; NRPBC = nitrate-reductase positive bacterial counts.
[b]GC = gastric cancers; B I = Billroth I gastrectomies; B II = Billroth II gastrectomies; NC = normal controls.

higher in partial gastrectomies than in normal controls (Table IV). Significantly higher mean levels of all these parameters were present in gastric cancers as compared to partial gastrectomies. When the groups of patients were compared, significant differences emerged (Table V). Only one gastric juice sample in normal controls showed bacterial contamination; none of the gastric juices from resected patients were sterile. NRPBC were cultured in the gastric juice of all resected patients except 3 BI samples and 2 BII samples.

No significant differences were observed between smokers and nonsmokers or between males and females.

TABLE VI. Histological Findings in Biopsies Taken Around the Stoma of Gastric Remnants in 45 Resected Patients

Histology	Billroth I (n = 23) no.	(%)	Billroth II (n = 22) no.	(%)	Chi-square test or Fisher's exact test (*)
Chronic superficial gastritis	20	(86.9)	17	(77.3)	ns
Cystic dilatations of glands	7	(30.4)	19	(86.4)	12.1; $P < 0.01$
Minimal atrophic gastritis	5	(21.7)	7	(31.8)	ns
Complete intestinal metaplasia	4	(17.4)	6	(27.2)	ns (*)
Diffuse atrophic gastritis	7	(30.4)	9	(40.9)	ns
Incomplete intestinal metaplasia	3	(13.0)	4	(18.8)	ns (*)
Mild dysplasia	2	(8.7)	2	(9.0)	ns (*)
H. pylori positive	13	(56.5)	12	(54.5)	ns

TABLE VII. Mean (± SD) Values for Parameters Measured in Two Groups of Gastroectomised Patients Subdivided According to Severity of Histological Changes

Degree	n	Patients Age	Years since surgery	pH	NO_2 (μmol/l)	NOC (μmol/l)	CEA (ng/ml)	TVC (log_{10} org./ml)	NRPBC (log_{10} org./ml)
Mild	17	50.8 ± 8.8 t = 0.57; ns	13.5 ± 3.2 t = 1.1; ns	6.6 ± 1.2 t = 0.58; ns	17.1 ± 15.1 t = −1.1; ns	0.026 ± 0.03 t = −4.2; p = 0.0003	18.1 ± 9.9 t = −2.76; p = 0.008	5.36 ± 1.9 t = −0.91; ns	4.34 ± 2.7 t = −1.2; ns
Serious	28	52.5 ± 10.7	12.5 ± 2.6	6.4 ± 1.1	22.2 ± 15.8	0.173 ± 0.18	33.5 ± 21.6	6.02 ± 2.5	5.3 ± 2.8

NO_2 = nitrite; NOC = N-nitroso compounds; CEA = carcino-embryonic antigen; TVC = total viable bacterial counts; NRPBC = nitrate-reductase positive bacterial counts.

Significantly higher mean (±SD) NOC levels were noted in *H. pylori*-positive BI patients (0.101 ± 0.10) compared with *H. pylori*-negative patients (0.011 ± 0.01) Z = −2.73; $P = 0.05$). Mean (±SD) NOC levels were also significantly higher in *H. pylori*-positive BII patients (0.235 ± 0.20) than *H. pylori*-negative patients (0.018 = 0.02) (Z = −3.07; p = 0.002). Mean (±SD) TVC were significantly lower in *H. pylori*-positive GC patients (5.38 ± 1.05) than *H. pylori*-negative patients (9.01 ± 0.91) (Z = −3.04; $P = 0.002$).

H. pylori were present in 16% of control biopsies, in 56% of BI, in 54.5% of BII, and in 43% of GC patients. The histological findings of biopsies taken around the anastomoses in resected patients are shown in Table VI. Cystic dilatation of glands was significantly more frequent in BII than in BI stumps. Biopsies were divided into two groups according to the seriousness of the histological changes and not according to the type of surgery. The relationship between the parameters of gastric juice and histological degrees is shown in Table VII. Mean NOC and CEA concentrations were significantly higher in the group with serious histologic changes.

The total number of bacterial stains isolated from 45 samples from resected patients and from 14 samples from gastric cancer patients was 181. Out of 181, 65 occurred in BI, 68 in BII and 48 in cancers. Figure 1 shows the proportion of gastric juices contamined by yeasts, aerobes, facultative anaerobes, and strict anerobes in BI, BII, and GC patients. The percentage of yeasts growing in the juice was significantly higher in GC than in the other two groups ($P = 0.01$). The percentage of juices contaminated by strict anaerobes was significantly lower in BI resections than in the other two groups ($P = 0.049$). In all the three groups the yeast contamination was always due to *Candida albicans*. Figure 2 shows the distribution

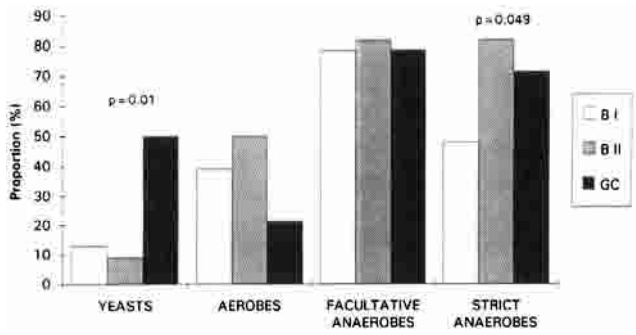
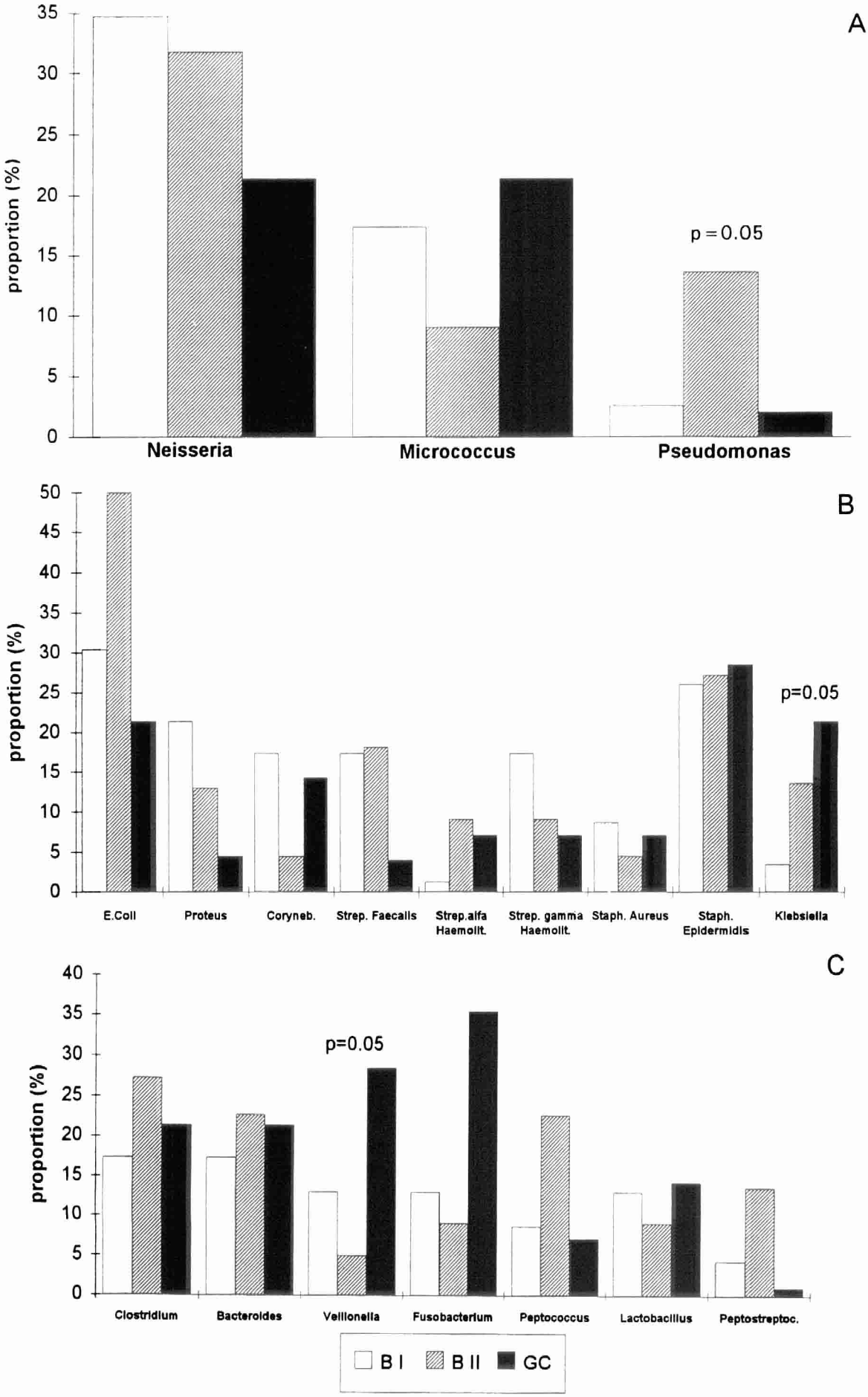


Fig. 1. Proportion of gastric juices contamined by yeasts, aerobes, facultative anaerobes, and strict aerobes in BI and BII partial gastrectomies and in gastric cancer patients. Acronyms: BI: Billroth I partial gastrectomy, BII: Billroth II partial gastrectomy, GC: Gastric cancer.

Fig. 2. Distribution of individual species subdivided in aerobes **(A)**, facultative anaerobes **(B)**, and strict anaerobes **(C)** in the gastric juices of both BI and BII partial gastrectomies and gastric cancer patients. Acronyms: BI: Billroth I partial gastrectomy, BII: Billroth II partial gastrectomy, GC: Gastric cancer.


A
proportion (%)
35
30
25
20
15
10
5
0
p = 0.05
Neisseria
Micrococcus
Pseudomonas
B
proportion (%)
50
45
40
35
30
25
20
15
10
5
0
p=0.05
E.Coli
Proteus
Coryneb.
Strep. Faecalis
Strep.alfa Haemolit.
Strep. gamma Haemolit.
Staph. Aureus
Staph. Epidermidis
Klebsiella
C
proportion (%)
40
35
30
25
20
15
10
5
0
p=0.05
Clostridium
Bacteroides
Veillonella
Fusobacterium
Peptococcus
Lactobacillus
Peptostreptoc.
B I
B II
GC

of individual species in the gastric juices of the three groups of patients. Among the aerobes, the proportion of juice samples contamined by *Pseudomonas spp.* was significantly higher in BII resections than in the other two groups ($P = 0.05$) and among the facultative anaerobes, contamination by *Klebsiella spp.* was significantly lower in BI resections ($P = 0.05$). *Veillonella spp.* growth, representing the strict anaerobes, was significantly lower in BII resections ($P = 0.05$). A significant relationship between NOC concentration in the gastric juice and facultative anaerobes was observed both in BI ($r_s = 0.590$; $P = 0.009$) and BII resections ($r_s = 0.481$; $P = 0.043$).

DISCUSSION

The results of this study support the Correa model of gastric carcinogenesis [15]. Furthermore, this is the first study that shows that CEA levels in gastric juice increase with elevated pH, nitrite, NOC concentrations, and bacterial counts. Previous studies have demonstrated a significant correlation between an increase in pH and an increase in bacterial counts in the gastric juice as well as in nitrite concentration of resected patients [3,14,16–20]. Comparing BI and BII reconstruction techniques, several reports [3,14,16,19] and the present study demonstrated that nitrite levels and bacterial counts are significantly higher in the gastric juice of BII patients. Considering all the resected patients, this study also showed a significant increase in pH of the gastric juice with the length of time since gastric surgery. However, this relationship was significant only in BII resections and not in BI. Since neither the mean age of BI and BII patients nor the mean age of BI and BII stumps differed significantly, it can be supposed that the correlation between pH and stump age does not depend on an increase in patient age but on specific factor(s) related to BII resectins, such as enterobiliary gastric reflux.

There are few published reports on gastric juice NOC assays in patients with partial gastrectomy for peptic ulcer [3,17,19–23]. Because of inadequate analitycal methods [24], the data reported by Schlag et al. [3] and Sturniolo et al. [21] are unreliable. Our data confirm previous observations [19,22] that correlate increased NOC levels with pH elevation. Such a correlation was not observed by others [17,20,23]. As already reported by Reed et al. [19], we also found significantly higher mean NOC levels in the gastric juice of patients with gastric resections as compared to normal controls. This was not observed by other authors [17,20,23].

Our study showed that both in BI and BII gastric juices, the NOC concentration increased with an increase in facultative anaerobic bacterial counts. Although no difference in facultative anaerobic counts was found between BI and BII juices, significantly higher mean NOC levels were found in the BII juices. This difference may be related to the significantly higher TVC, NRPBC, and strict anaerobic counts detected in these juices. Our observations showed that in the gastric juice of BII patients, a marked increase in pH caused a significant decrease in NOC levels. This could be explained by the finding that a pH >7 may promote the growth of bacterial species capable of degrading NOC [24,25]. However, it is more likely to be due to the increased decomposition effect of sulphamic acid at a higher pH [26,27].

CONCLUSIONS

This study has confirmed that progressive hypochlorhydria, growth of nitrate-reducing microflora, and formation of NOC play a very important role and have a synergic action in cancerogenesis of the gastric remnant in patients operated on for benign peptic ulcer disease. The demonstration that NOC levels in the gastric juice were directly and significantly correlated to the seriousness of the histological changes in the gastric stump supports this conclusion.

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